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Blood Group ABO, Placental Malaria Parasitization and Pregnancy Outcome in Munuki Primary health Centre in South Sudan

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Abstract

Several studies in Malaria endemic areas have reported conflicting observations on Blood Group ABO association with placental Malaria and pregnancy outcome. The purpose of this study is to determine the association between Placental Malaria and Blood Group O in the Munuki Primary Health Care Centre in South Sudan.

Material and Methods: This was a prospective study. Each pregnant lady was enrolled after signing of informed consent and meeting study entry criteria. In all 589 pregnant women were evaluated. For each done were; ABO phenotype ones, at the enrolment and testing for malaria in and twice in the cause of pregnancy. Peripheral blood malaria parasites testing were by using RDT test to describe the type of plasmodium species.

Results: The 589 enrolled were as follows; 154 (32.6%), 124 (26.3%) and 182 (38.6%) primigravidae, secundigravidae and multigravidae, respectively. The ABO distribution was; 124 (A), 59 (B), 14 (AB) and 257 (O). Negative peripheral Malaria parasites for pregnant women at ANC have higher positive Peripheral Malaria at DR with OR 2.93 CI (1.78__4.88) and ANC positive Malaria cases had higher chances of become positive during delivery OR 0.43 CI (0.30__0.62). Blood group O and peripheral malaria at DR with OR 1.17 CI (0.92__1.48) and Other Blood group at DR with OR 0.84 CI (0.67__1.06). Blood group O participants had higher P. Falciparum 60 /106 accounts for 56.6%. These results revealed significance interaction effect of Placental Malaria Parasites in Blood group O in Munuki Primary Health Center in Juba-South Sudan. This study recommends usage of protective measures against malaria during pregnancy.

Keywords: Pregnancy; Placental Malaria; Blood group A B O

Introduction

Majority of studies in many endemic areas have recorded differences in Blood group ABO presentations among placental malaria infections regardless of age, parity and type of Plasmodium species. Five species of malaria parasites cause disease to human population specifically targeting pregnant women; P. falciparum (Welch), P. vivax, (Grassi

& Feletti) P. Ovale (Stephens), P. malariae (Laveran) and P. Knowlesi [1]. It is also apparent that population movements affect exposure to malaria. In this study, a clear knowledge of both Internal displaced persons and resident's susceptibility to placental malaria infections through examining the blood group ABO and malaria presented opportunity for understanding malaria endemicity and displacement patterns to help in pregnancy associated Malaria control programs within the study population. For example, malaria accounts for 2—15% of maternal anaemia and 5—14% of low birth weight [2]. Moreover, malaria causes 30% of preventable low birth weight; 3—5% all new born deaths [3].

In many previous research studies recorded that some red blood cells variants are related to ABO system and genetic modulations do affect specifically blood group O1. Furthermore, protective Heamatological factors such hemoglobin's S, C and E, α and β thalassemia, Glucose-6-phosphate dehydrogenase deficiency, (SAO) Southern Asian ovalocytosis, and Glycophorins A, B and C variants, all of which some has effects in Placental malaria parasites pathophysiology [4]. Blood group A was reported to be associated with severe malaria disease in general population [5], and has been linked to P. Falciparum resetting [6], whereas blood group O was suggested to play role in protection against severe Malaria disease [7].

This was designed to follow pregnant mother from time of first reporting to antenatal clinic (ANC) through gestation till delivery. The documented were status positive or negative of malarial, placental malaria parasites antigens and other factors (parity, age of the mother, Placental parasites, placental weight and baby birth weight) that could play role on Malaria control programs as per Malaria Roll Back strategy. We present the first report to explain whether there is an association between ABO phenotypes and placental malaria Parasitization in South Sudan.

Materials and Methods

Study Population

The study was done from June 2017___ May 2018 at Munuki Primary Health Care Payam Unit, Juba South Sudan located Western part of Juba town approximately 3km. Malaria cases are highly reported in this area and yearly transmission intensifies during the rainy season from June to October. Moreover, the study site represents central suburb area with different internal displaced communities as per Central Equatoria Government decision and approval to conduct a research in the area because the endogenous communities receive free medical services from WHO, UNICEF and others; ABO normal distribution among the communities ranges from 45% O and 55% others blood groups.

Study Entry

- **Inclusion:** Confirmed pregnancy and from South Sudanese's origin.
- **Exclusion:** Participants with emergency obstetric problems (PET, obstetric bleeding, obstructed labor or sepsis) or with recent blood transfusion were excluded.

Procedure

Participants were approached at the first ANC attendance and during obstetric labor and informed consent was requested. A preformed questionnaire was completed by a trained midwife requesting information on age, parity, obstetric history, bed net and antimalarial use. Subsequently a total of 589 who attended the Primary Health Care unit ANC services were enrolled and progressively followed up till delivery.

Training Workshop for Data Collectors and Research Assistants

A three days training workshop for data collectors was completed at College of Nursing and Midwifery from 15/July _18 July 2016 with a total of eighteen health professionals from two Medical Officers, four Laboratory Technicians, six Midwives and six Nurses. The purpose of the seminar was to explain the techniques and research protocols. A one-day pilot study was done at Gueri primary health center six km far from the study site to measure the data collector knowledge on performing the research efficiently and correctly.

Rapid Diagnostic Test for Malaria Parasites (RDT)

We applied (RDTs) in cassette format and gave results in less than 20 minutes. A capillary blood specimen taken from the finger of the study participant was put to the sample pad on the test card along with a buffer solution specifically for Malaria test. The approved CE (European) RDTs for use in the malaria-endemic communities that had three types of malaria antigens; the histidine-rich protein 2 (HRP-2) from Plasmodium falciparum and parasite-specific lactate dehydrogenase (pLDH) or Plasmodium aldolase from the parasite glycolytic pathway found in all Plasmodium species.

This study interpretations in this way; Positive RDT means both control and Test lines are visible clearly while Negative RDT means only Control line is visible; but invalid results mean Test line is visible without appearance of

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Control line. For Plasmodium species results interpretations; both two lines Control and Test seen above that means a Plasmodium Falciparum was detected while the two lines (control & Test) seen one above and one down means it is any of Other Plasmodium species.

Pregnant Women in Antenatal Clinic (ANC)

During ANC routine services, a follow up study was conducted to pregnant women who attend regular antenatal clinic at Munuki Primary Health Care. After receiving a signed consent form, A confirmed pregnant woman with negative HIV record card is asked to fill in a structure questionnaire, donating 1ml of capillary blood for RDT to detect peripheral malaria parasites antibodies particularly Histidine Rich Protein 2 (HRP2) and agglutination Test for ABO identification applying commercial antisera (Biotech Laboratories Ltd., Ipswich, and Suffolk, UK) and finally, telephone number for follow up communications in case of any obstetric or medical complications.

Pregnant Women in Delivery Room (DR)

After normal delivery or preterm labor, a second consent form for second 1ml of capillary blood for RDT to screen peripheral malaria parasites during delivery or preterm labor, Placental weight and final Baby weight. Each Twin delivered babies had its own study number as well as two placentae have different weighting number. Following delivery of the placenta, a placental blood sample was obtained by a fine needle aspiration and placed on parasite-specific lactate dehydrogenase (pLDH) to screen Placental malaria parasites antigens and type of malaria species.

Categorical variables were investigated using the chi-square or odds ratios and their 95% confidence intervals assessed. Variations in means calculations were assessed by ANOVA for normal distribution data. Multiple linear regressions were introduced to analyze other associated risk factors in pregnancy outcomes. Factors with a significance level of $p < 0.05$ were included in the model. These were: mother age, number of pregnancies, and ABO phenotype.

Statistical Method

SPSS statistical software version 21 was used after data cleaning and double-checked before analysis. ANOVA and Student T-Test were applied to compare variables means for both normality in distributions. Mann-Whitney Test to check abnormal distributions among the variables. Furthermore, Fisher exact test to rule out data appropriateness and liner regression to crosscheck other influencing factors. Maternal Blood group O versus Other Blood groups. A P-value of <0.005 was used to measure the variables associations together with Confidence Interval 95% and an Odd Ratio > 1.1 or 0.1

Data Quality Control

For raw data accuracy, the research assistant applied standardized weighting scale machines to avoid measurements misclassifications for both baby birth and Placental weight. A secondary check for was done at National Blood Bank Laboratory. At the College of physicians and Surgeons a seminar on preliminary study results was initiated in October 2019 attended by senior College teaching staff and Ministry of Health Directors to give inputs and comments on data whether meeting intended purpose or not. Moreover, data completeness exercises to reduce or minimize missing value.

Ethical Clearance

The research protocol and consent proposal were approved by IEC at MOH, memo number No. MOH-SSD, 01 dated 21-07-2015 for study to be conducted and again obtaining an approval on publication with Ref: MOH/RERB/P/003/202.

Results

Characteristics of Pregnant Women

A total of 589 deliveries documented. Of these, 22 were twins, 19 abortions, 49 referrals, 31 home deliveries and 18 missing and 463 singleton deliveries. The data of placental malaria parasites, placental and birth weights were complete in 472 women; these data were included in the final analyses. Out of these 472 women, there were 154 (32.6%), 124 (26.3%) and 182 (38.6%) primigravidae, secundigravidae and multigravidae, respectively as per Table 1. The mean (SD) age was 24.3 (6.7) years. Of the 450 women, 257 (54.4%), 124 (26.3%), 59 (12.5%) and 14 (3.0%) had blood group O, A, B and AB, respectively.

The mean birth weight was 3.46kg while the Placental weight means 612.5 gm. Standard deviation for both placental and baby were 126.3 and 13.97 respectively. 102/468 (21.7%) were Positive for peripheral malaria parasites during enrollment and 366 were negative of which 87 were positive peripheral malaria parasites during delivery times. Those Pregnant women with blood group O reported possessing 6 months WHO treated bed net 79/152 (51.9%), those pregnant women reported possessing 3months WHO treated bed net 39/152 (25.6%), those pregnant women reported having UNICEF treated bed net 85/152 (55.9%), those pregnant who reported having local made untreated bed net 23/152 (15.1%) and finally pregnant women reported haven't bed net 25/152 (16.4%). Pregnant women

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with Blood group O reported to sleep alone inside treated bed net 42/245 (17.1%), Blood group O reported to allow husband sleep alone inside bed net 11/245 (4.4%), Blood group O reported to share bed net with husband 64/245 (26.1%) and blood group O reported to share bed net with children 128/245 (52.2%) 148/239 (61.8%) Internal displaced pregnant women have Blood group O, 91/239 (38.1%) Among residents with Blood group O, 73/118 (61.8%) Internal displaced mothers with blood group A, 45 (38.1%) residents' mothers with blood group A, 28(50.9%) Internal displaced mothers with blood group B, 27 (49.0%) resident mothers with blood group B and finally 9 Internal displaced with blood group AB and 5 residents with blood group AB.

Prevalence of Placental Malaria

Distribution of Plasmodium species among all blood groups whether internal displaced women or residents appeared like this; P. Falciparum 60/106 (56.6%) cases for Blood group O, 28/106 (26.4%) cases for blood group A, 10/106 (9.4%) cases for Blood group B, 4 cases for blood group AB and 4 cases for other negative ones. P. Vivax revealed 7 cases for blood group O, 4 cases for blood group A, one case for blood group AB. P. Ovale only one for blood group O. While P. Malariae showed three cases from blood group O and one case for AB-ve. For mixed infections only, blood group A showed one case. Negative Peripheral Malaria parasites for pregnant women at ANC have higher Positive Peripheral Malaria at delivery room (DR) with OR 2.93 CI 95% (1.78__4.88) and ANC positive Malaria cases have higher chances of become positive during delivery OR 0.43 CI 95% (0.30___0.62). At the enrollment those who are negative are more likely to develop Placental Malaria parasites OR 1.34 CI 95% (1.13___1.60) as per Table 2.

Placental Malaria Parasitization and Blood Group O

Blood group O and peripheral malaria at DR with OR 1.17 CI 95% (0.92___1.48) and Other Blood group at DR with OR 0.84 CI 95% (0.67___1.06) as per Table 3. Blood group O has higher chances for sustaining positive peripheral Malaria parasites with OR 2.9 CI 95% (1.7__4.8) as per Table 4.

		Placental Malaria Parasites						Total
		Negative	P. Falciparum	P. Vivax	P. Ovale	P. Malariae	Mixed	
ABO	O	185	60	7	1	3	0	256
	A	91	28	4	0	0	1	124
	B	48	10	1	0	0	0	59
	AB	9	4	1	0	0	0	14
	O-ve	5	2	0	0	0	0	7
	A-ve	2	1	0	0	0	0	3
	B-ve	5	1	0	0	0	0	6
	AB-ve	1	0	0	0	1	0	2
Total		346	106	13	1	4	1	471

Table 1: The data of placental malaria parasites.

Comparison	Odd Ratio	95% Confidence Interval	
		Lower	Upper
Odds Ratio for RDT Recruit (Negative / Positive)	2.953	1.783	4.889
For Cohort RDT Del = Negative	1.283	1.111	1.481
For Cohort RDT Del = Positive	0.434	0.3	0.629
N of Valid Cases	468		

Table 2: Prevalence of Placental Malaria.

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Blood Group		B	Std. Error	Wald	df	Sig,	OR	95% Confidence Interval for Exp(B)	
								Lower Bound	Upper Bound
O+NV		5.073	1.013	25.078	1	0			
	Placental Parasites	-0.012	0.238	0.003	1	959	988	0.619	1.576
A+ve		4.387	1.016	18.641	1	0			
	Placental Parasites	-0.009	0.239	0.002	1	968	0.991	0.62	1.582
B+ve		3.814	1.021	13.953	1	0			
	Placental Parasites	-0.037	0.24	0.024	1	877	0.964	0.602	1.543
AB+ve		2.596	1.047	6.146	1	0.013			
	Placental Parasites	-0.065	0.247	0.07	1	0.792	0.937	0.577	1.521
0-ve		1.162	1.156	1.012	1	0.314			
	Placental Parasites	0.079	0.269	0.087	1	269	1.083	0.639	1.834
A-ve		0.22	1.355	0.026	1	0.871			
	Placental Parasites	0.018	0.317	0.003	1	0.954	1.019	0.547	1.896
B-ve		0.9	1.196	0.567	1	0.452			
	Placental Parasites	0.079	0.278	0.081	1	0.776	1.082	0.628	1.866

Table 3: Blood group O and peripheral malaria.

ABO		Odd Ratio	95% Confidence Interval	
			Lower	Upper
O	Odds Ratio for RDTDel (Negative / Positive)	2.947	1.436	6.048
	For cohort RDTRecruit = Negative	1.347	1.046	1.735
	For cohort RDTRecruit = Positive	0.457	0.283	0.738
	N of Valid Cases	254		
A	Odds Ratio for RDTDel (Negative / Positive)	2.118	0.792	5.661
	For cohort RDTRecruit = Negative	1.194	0.91	1.567
	For cohort RDT Recruit = Positive	0.564	0.274	1.159
	N of Valid Cases	124		
B	Odds Ratio for RDT Del (Negative / Positive)	4.1	0.899	18.698
	For cohort RDT Recruit = Negative	1.506	0.829	2.737
	For cohort RDT Recruit = Positive	0.367	0.14	0.966
	N of Valid Cases	58		
AB	Odds Ratio for RDT Del (Negative / Positive)	1.667	0.195	14.266
	For cohort RDT Recruit = Negative	1.25	0.477	3.276
	For cohort RDT Recruit = Positive	0.75	0.226	2.491
	N of Valid Cases	14		
O-ve	For cohort RDT Recruit = Negative	2	0.5	7.997
	N of Valid Cases	7		
A-ve	Odds Ratio for RDTDel (Negative /Positive)	.[a]		

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B-ve	Odds Ratio for RDTDel (Negative / Positive)	1	0.034	29.807
	For cohort RDTRecruit = Negative	1	0.183	5.46
	For cohort RDTRecruit = Positive	1	0.183	5.46
	N of Valid Cases	6		
AB-ve	Odds Ratio for RDTDel (Negative / Positive)	[b]		
Total	Odds Ratio for RDTDel (Negative / Positive)	2.953	1.783	4.889
	For cohort RDTRecruit = Negative	1.349	1.132	1.606
	For cohort RDTRecruit = Positive	0.457	0.325	0.641
	N of Valid Cases	468		
a. No statistics are computed because RDTDel and RDTRecruit are constants.				
b. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.				

Table 4: Peripheral Malaria parasites with OR.

Discussion

Previous studies have linked blood groups with proclivity and intensity of malaria parasitisasion. Blood group system particularly Blood group ABO status is more studied than others1. In the current study the overall prevalence of Placental Parasitization is 22.5% at Munuki Primary Health Care Center. This is higher than 19.9 % Placental Malaria reported in Cameron and lower than 65.2% reported in Nigeria [7] and 58.9% recorded in Sudan [8]. These Placental Malaria Prevalence variations could be because of geographical locations endemicity patterns and infected Mosquito vector bites intensity. This study showed significance relationship between positive peripheral malaria parasites at delivery and Placental Malaria Parasitization with OR 0.43 CI 95% (0.30__0.62) similarly to study conducted in Kenya where OR 2.11 CI 95% (1.47_3.04)14. It was observed the significance association between having Positive peripheral Malaria parasites during enrollment and blood group O OR 0.45 CI 95% (0.32__0.64).

These differences associated with multigravidae could be explained as a parity-specific factor of blood group O phenotype and its protective malarial immunity that occurs in multigravidae. Moreover, weak rosette formation could assist in development of innate resistance to P. falciparum malaria which occurs in some special red cell disorders and particularly those of blood group O phenotype. This could explain parity specific immunity to placental malaria in women with the O phenotype 1.

Sharing Mosquito bed net at night during sleeping time was significantly related to Placental Malaria Parasitization with OR 1.98 CI 95% (1.12__3.49) similarly to study documented in Cameroon with RR 0.82, 95% CI 0.69–0.98)12.

P. falciparum infections in pregnancy has different presentations within parity groups, then strengthened parity-specific immunity in multigravidae with the O phenotype must connect to improved absent of placental malaria parasites. Factors influencing cytoadherence and placental malaria parasites sequestration are of great value since infected red blood cells bearing the variant surface antigen, VAR2CSA that adheres to the glycosaminoglycan chondroitin sulphate a seen on the surface of syncitiotrophoblast [9]. P. falciparum changes composition of the erythrocyte membrane such as glycophorin band 1 and spectrin playing a part in antigenicity and being dispersed by the reticuloendothelial system [10-15].

A study in Sudan reported connection between blood group O and Placental malaria infections15 while a research results in Ghana documented elevated in prevalence of placental malaria among blood group O in pregnant women. Despite it has been accepted that blood group O exposure to malaria parasites during pregnancy creates susceptibility of placenta cells to malaria infection; other factors underline factors may increase liability of blood group O to placental malaria infections. The well-known factors globally are (a) blood group O geographical distribution in tropic and subtropical areas (stable and non-stable areas); (b) A faith that; Plasmodium falciparum has affected the human genome structure, (c) the linkages of blood group O and clinical outcome of malaria (d) finally the cytoadhesion of infected RBCs and parasite invasion mechanism is considered to play role18 [15-19].

This study results had encountered some limitations during designing phase where civil war broke out in the initial approved study locations in Northern part of the Country in 2014 followed by massive internal displacement in 2015 for Southern part of the country and logistic factors within

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the country. Rapid diagnostic Test kids were not superior to Traditional Microscope and RT-PCR; the study didn't address pathological classifications of Placental Malaria from present, past and chronic infections within placental microvascular tissues and placental villi.

Conclusion

This study has established that there is relationship between placenta malaria parasitaemia and outcome of pregnancy. In addition this opens avenues and appetite for South Sudanese academicians, policy-makers and decision makers at National Malaria Control program for the first time ever to draw more research in Science of Placental Malaria and ABO system for South Sudanese population. This 22.5% prevalence of Placental Malaria in Munuki alone could be generalized to other areas of the Country because of similarities of ecological and transmissibility patterns of Malaria.

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